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Commentary

Impact of vaccination during pregnancy on infants' immune responses to vaccinations- definitions and statistical approaches

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1. Introduction

Vaccination during pregnancy is a proven strategy to protect infants against infectious diseases in early life, and is currently successfully implemented for tetanus, pertussis, influenza and COVID-19 in many countries. Yet, vaccination during pregnancy modifies the humoral immune response of infants to their own vaccinations after both primary and booster vaccination [1–4]. After tetanus-diphtheria-acellular pertussis (Tdap) vaccination during pregnancy, lower antibody levels have been reported for some vaccine antigens (e.g. pertussis toxin, filamentous hemagglutinin, pertactin, diphtheria), as well as antibodies against vaccines' conjugated to carrier proteins (e.g. pneumococcal vaccines conjugated to diphtheria toxoids [DT]) in infants born to vaccinated compared to unvaccinated women (termed as "interference" or "blunting") [5,6]. In contrast, Tdap vaccination during pregnancy is also associated with higher anti-polyribosyl ribitol phosphate (PRP) and anti-tetanus toxoid (TT) antibody titers in offspring of vaccinated compared to unvaccinated women [1,3,7,8]. The latter modification of the infants' immune response is not accurately described by the use of the term "blunting".

The terms "modification" of immune responses, "interference" and "blunting" are used interchangeably in the scientific literature. However, modification of immune responses and interference are general terms that describe the influence of vaccination during pregnancy on the immune responses of the infant. Thus, these terms could be used regardless of whether vaccination during pregnancy enhances or reduces the immune responses in infants. Blunting of the immune response is a term that implies reduced immune responses in infants born to vaccinated compared with unvaccinated women and should thus only be used to describe a reduced immune response.

The lower antibody levels observed after infant vaccination for some antigens could lead to an increased risk for certain infectious diseases in infants born to vaccinated women. However, the clinical relevance of blunting should come from appropriately designed surveillance and/or epidemiological studies evaluating disease incidence rates, severity of infection and/or hospitalization rates comparing infants born to vaccinated and unvaccinated women. This approach requires comprehensive surveillance systems in place, which can be a challenge. For pertussis for example, current evidence does not suggest that infants born to women vaccinated against pertussis during pregnancy have higher risk for pertussis later in their infancy compared to infants of unvaccinated women [9]. For more prevalent infections, evidence for clinical significance of blunting may also be obtained from large-scale clinical trials.

As investigating the clinical significance of the effect of vaccination during pregnancy on infants' immune responses is challenging, research has focused on immunogenicity studies to assess potential modifications of infants' immune responses after vaccination during pregnancy. However, despite years of research, there are substantial variations in the statistical approach taken to explore this phenomenon. This variation has the potential to lead to erroneous conclusions. In this commentary, we present and dis-

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cuss several potential statistical approaches to investigate this phenomenon, and highlight their advantages and drawbacks.

2. Impact on infants' immune responses- statistical approaches

2.1. Geometric mean concentrations (or titres) of antigen-specific antibodies

The most commonly used approach to investigate the impact of in-pregnancy vaccination on humoral immune responses is the comparison of antigen-specific antibody levels (or titers) in infants born to women vaccinated during pregnancy with those born to unvaccinated women. The strictest criterion to confirm lower immune responses is a statistically significant lower geometric mean concentration (GMC) (or geometric mean titer [GMT]) of antigen-specific antibodies in infants of vaccinated mothers after primary and/or booster immunization with no overlap in the bounds of the confidence intervals (CI) in the two groups. However, this is sometimes difficult to achieve given the small sample size of most maternal vaccination studies. Therefore, a statistically significant difference, even with overlap in the bounds of the CI is an acceptable and still commonly used criterion.

2.2. Geometric mean ratio of antigen-specific antibodies

Another approach to describe possible modifications of the humoral immune response is to derive the geometric mean ratio (GMR) of antigen-specific antibody levels (or titers) of infants born to vaccinated versus unvaccinated women after primary or booster vaccination, and this approach has been recently used in maternal pertussis vaccination studies [7,10]. A GMR and an upper bound of the GMR CI below 1 implies significantly lower antibody levels (or titers) in infants born to vaccinated compared with unvaccinated women, and thus supports reduction in immune responses. Regression models (e.g. mixed effects model) are robust statistical tools to derive this ratio, with the advantage of adjustment for co-variables that could affect the infants' immune responses, such as gestational age, sex, preterm vs term, breastfed vs formula fed, maternal health status and previous infections and vaccinations [11].

2.3. Fold-change in antigen-specific antibody levels (or titres) post vs. pre -vaccination

The fold change in antibody levels (or titres) post vs. pre -vaccination reflects the magnitude of changes in vaccine-induced antibody responses relative to baseline. This fold change measure could also be used to define seroconversion. In subjects with pre-vaccination antibody levels above the lower limit of quantification (LLOQ) of the laboratory assay, seroconversion is commonly defined as a predefined fold-increase in antibody levels [12]. In subjects with pre-vaccination antibody levels below the LLOQ of the laboratory assay, seroconversion is defined as quantifiable antibody levels post-vaccination, or two-fold increase or more above the LLOQ [12]. A significant drawback of this approach in the context of in-pregnancy vaccination is that infants' pre-primary vaccination antibody levels are maternally derived while post-vaccination antibody levels are mainly produced by the infant, thus the fold change does not accurately reflect the infants' immune responses to vaccination. In addition, the fold change measures the relative increase to baseline and is thus largely influenced by baseline antibody levels, which are expected to be lower in infants born to unvaccinated women, making any change in antibody levels greater in those infants. Given those limitations, using the fold change or seroconversion rates is not an ideal approach to compare between infants born to vaccinated versus unvaccinated women to define modification of immune responses after primary vaccination.

2.4. Seroprotection rates for specific diseases

The above-mentioned approaches do not take into account whether a reduction in antibody levels is associated with a reduction in protection against specific diseases. Investigating whether a reduction in antibody levels is associated with a potentially higher risk of infection is feasible for diseases for which a correlate of protection [COP] exists. Examples include tetanus (anti-TT IgG > 0.1 IU/ml), diphtheria (anti-DT IgG > 0.1 IU/ml), and *Haemophilus influenzae* type b (Hib) diseases (anti-PRP IgG > 0.15 ug/ml after vaccination with conjugated Hib polysaccharide vaccine and anti-PRP IgG > 1 ug/ml after vaccination with unconjugated Hib polysaccharide vaccine) [13]. A statistically significant reduction in seroprotection rate in infants born to vaccinated compared with unvaccinated women might also be used to define interference. However, this approach is not possible for vaccination against diseases for which a well-defined COP does not exist (e.g. pertussis).

3. Concluding remarks and future directions

There are various statistical approaches that could be applied to investigate modifications of humoral immune responses in infants after vaccination during pregnancy (Table 1). These different approaches have already been used variably in published studies on Tdap vaccination during pregnancy (Table 2). The use of GMR has the advantage for adjusting for co-variates that affect the humoral immune responses and thus should be preferably presented in future studies. Seroprotection rates for diseases that have COP against should also be compared between infants born to vaccinated versus unvaccinated women. Combining different statistical approaches might increase the confidence in conclusions about the existence, direction and the degree of this modification. In addition, it is also important to assess the quality of humoral immune response and the cellular mediated immune responses generated after infant vaccination and compare it between infants born to vaccinated and unvaccinated women. Immunogenicity results need also to be coupled with clinical data to better inform public health policy makers about the true significance of any modification in the settings of maternal immunization programs.

Author contributions

Prof. Dr. Kirsten Maertens and Dr. Bahaa Abu Raya drafted the commentary and all other authors performed a critical revision of the commentary.

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Declaration of Competing Interest

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Table 1
Potential statistical approaches to investigate modifications of humoral immune response to vaccinations in infants after vaccination during pregnancy.

Statistical approach	Advantages	Disadvantages
Immunological modifications		
Levels of antigen-specific antibodies*	-Simple statistical approach.	- Clinical significance unclear as it is not assessed against correlate of protection.
Geometric mean ratio of antigen-specific antibodies*	-Possibility of adjustment for co-variates that could affect immune response.	- Needs well-trained statisticians. - Clinical significance unclear as it is not assessed against correlate of protection.
Fold-change in antigen-specific antibody levels post- vs. pre – vaccination*	- Could be used to determine seroconversion rates.	- Requires both pre and post -vaccination antibody levels. - Not a true fold change for primary vaccination since you measure the change from maternal to infant antibodies.- Needs to be carefully interpreted as higher fold changes are expected if baseline antibody levels are low (as in the case of infants born to unvaccinated women) . - Seroconversion rates definition varies and affected by baseline antibody levels. - Clinical significance unclear as it is not assessed against correlate of protection.
Seroprotection rates*	- Simple statistical approach. - Provides more insights into clinical relevance.	- No adjustment for co-variates that could affect immune responses. - Some pathogens don't have well-defined correlate of protection.
Clinical significance		
Increase in disease incidence, severity or hospitalization rates*	-Most accurate indicator of immunological modifications.	- Needs large-scale surveillance data that spans for long years after implementation of maternal vaccination program. - Might be difficult to determine for relatively rare diseases and diseases for which less severe presentations could be under-diagnosed and under-reported.

* In infants born to vaccinated compared with unvaccinated mothers.

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Table 2
Statistical approaches used in different studies to investigate modifications of humoral immune response to infant vaccination after Tdap vaccination during pregnancy.

Author	Geometric mean concentrations (or titres)	Geometric mean ratio	Seroprotection rate
Hardy-Fairbanks et al [14]	✓		✓
Ladhani et al [8]	✓		✓
Hoang et al [15]	✓		
Maertens et al [16]	✓		
Maertens et al [17]	✓		
Maertens et al [18]	✓		
Munoz et al [3]	✓		✓
Maertens et al [5]	✓		✓
Halperin et al [4]	✓		✓
Barug et al [10]	✓	✓	
Zimmermann et al [7]	✓	✓	✓
Rice et al [6]	✓		✓
Barug et al [19]	✓	✓	✓
Klein et al [20]	✓		✓
Perret et al [21]	✓		✓
Wanlapakorn et al [22]	✓		
Wanlapakorn et al [23]	✓		✓

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